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INVERTEBRATE BIODIVERSITY (ANTS, BEES, AND OTHERS) ASSOCIATED WITH STEM DOMATIA OF THE INDIAN MYRMECOPHYTE *HUMBOLDTIA BRUNONIS* WALLICH (MAGNOLIOPHYTA: FABACEAE)

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Abstract.—The legume tree genus *Humboldtia* Vahl contains three mutualistic, myrmecophytic species. These trees develop inflated, hollow internodes which differentiate a single, self-opening entrance hole for each domicile unit. At leaf flush, hundreds of new, accessible, empty domatia are available to any organism capable of entering and defending the hollow space. In terms of abundance, ants are the primary occupant of the hollow internodes of *H. brunonis* Wallich of India, followed by a bee, *Braunsapis* sp., and several minor inhabitants including an arboreal annelid. *Humboldtia laurifolia* Vahl in Sri Lanka is the closest relative of *H. brunonis*, and we compare and discuss the internode inhabitants of these two geographically separated, but morphologically very similar tree species.

Key Words: *Humboldtia brunonis*, *Humboldtia laurifolia*, ants, *Braunsapis*, mutualism, India, Sri Lanka

Numerous plants have entered into mutualistic relationships with ants. The plant evolves either, or both, a solid or liquid food supply, and a domicile in the form of leaf pouches, inflated leaf petioles, or a hollow stem, while the ants provide a modicum of anti-herbivore defense. The simplest arrangement is where a plant possesses extrafloral nectaries which are visited by an assortment of ants, wasps, and other invertebrates to obtain nectar from these secretory tissues (Kopter 1991, Rickson and Rickson 1998). In these "open" systems there is usually no dominant ant species and the visiting species assemblage can change over time. Those plants possessing a predictable domatia, or a food source requiring some innate ability to access, often have a particular ant species dominating the plant, although this too can change through time, perhaps through simple competition (Maschwitz et al. 1991), or seemingly at a particular age stature of the plant (McKey 1991). Often those ants which are restricted to a particular plant species tend to be very aggressive toward intruders, and the plants, conversely, can have lower innate chemical defenses (Rehr et al. 1973). The actual success of the protection seems to vary widely from system to system, but where measured, a positive benefit to the plant usually arises from the relationship.

Little information is available on the total complement of organisms living within a plant's domatia, across a series of populations, because the ants dominating the bio-

mass of associated organisms are usually collected and reported as the animal partners involved (Huxley 1978).

In a number of relationships, the ant, often a founding queen, chews a hole into a preformed, hollow domicile. In these situations, there is always an ant present within the chamber, the queen and/or her brood, should another organism seek to occupy the same space. Another group of plants also develops preformed domatia, but the plant provides a self-opening entrance hole to each of the chambers. If there is already an ant colony present on the plant, workers can move eggs or brood into newly available chambers as they become available. Ants are always dominant in these situations. However, in the case of a new leaf flush where each leaf is associated with an adjacent stem domatium having an open entrance hole, there may be a superabundance of open potential nest sites available to any organism capable of accessing the hole first, without any predisposed ability to find a specific location or method of chewing into the plant tissue. In such cases, other organisms may also evolve a predisposition to regularly take up residency in the hollow domatia and either compete with ants for dominance, or coexist.

Humboldtia, with four species in southern India and one species in Sri Lanka, has three species which develop extrafloral nectaries on the leaf blades, leaf stipules, and flower buds, and domatia in the form of inflated, hollow stem internodes (Krombein et al. 1999). Each internodal domatium has a single, consistently placed, self-opening entrance hole. Potential occupants of the hollow internodes do not need any special location or chewing instincts, or special mouth parts, to gain access to the open cavity.

The node between each hollow internode along a branch is solid. This creates a linear series of independent units, each with a preformed access opening. A shoot, which has an average of eight to ten new leaf/internodes associated with each stem flush, produces a similar number of individual, potential nest sites with each leaf flush. An annual production of 20–30 new shoots produces a corresponding 200–300 new potential nest sites, each year, available to any organism capable of accessing the entrance hole and defending the hollow internode space. The ant-associated *Humboldtia* species are gregarious with as many as 100+ individuals growing in close proximity, and so the yearly leaf/domatia production produces a very large number of potential nesting sites within a small area.

Humboldtia laurifolia Vahl, endemic to the southwestern lowland wet zone of Sri Lanka, has been thoroughly sampled and its internode-inhabiting associates enumerated (Krombein et al. 1999). The inhabitants include: 14 species of ants, four wasps, one bee, two dipterans, three coleopterans, a pseudoscorpion, and an annelid. Included in this list are seven undescribed species and a number of first and second order predators and parasites on the various inhabitants. Finally, the cryptic nature of the *Humboldtia* associates can be appreciated by noting that the Smithsonian Institution carried out a Fauna of Ceylon project over 12 years and yet the inhabitants of this common tree largely escaped detection and collection.

Humboldtia brunonis Wallich, is a small, gregarious, understory tree found along the eastern edge of the Western Ghats in southern India from about 13°70′N, 75°00′E to 11°30′N, 76°30′E (Ramesh et al. 1997). This species, along with *H. decurrens* Beddome ex Oliver found in the southwestern part of the Western Ghats, possesses the same extrafloral nectary placement and domicile traits as described for *H. laurifolia* (Krombein et al. 1999).

Materials and Methods

Several hundred hollow internodes from random trees of *H. brunonis* growing in the Makut Reserve Forest, Coorg District, Karnataka State, India (12°10′N, 75°50′E) were first collected by KG in 1994, with author KVK making a second collection effort

within this population of trees in 1997. The Makut population is a little south of the center of the North-South distribution of the species (Ramesh et al. 1997). The internodes were split open, distribution of the included organisms noted, and samples preserved in 70% alcohol. Only those organisms actually collected from within the hollow internodes, as opposed to specimens found foraging on the tree, were included in this summary.

Results

We present below, in general order of abundance, the invertebrates collected from *Humboldtia brunonis* hollow internodes.

Hymenoptera

Formicidae

Krombein collected 100 hollow internodes of which 27 contained ants, while another 38 were empty, 13 contained a common bee *Braunsapis* sp. (see below), and the remaining internodes possessed an assortment of organisms. Eleven ant species were found nesting within the hollow stem internodes. There was no single dominant ant taxon although *Crematogaster* spp. were present in approximately 50% of the ant-occupied internodes, and the three species within that genus contributed approximately equally to that 50%. A surprising finding was the lack of weaver ants, *Oecophylla smaragdina* (F.) associating with *H. brunonis* at Makut. This species is a common visitor to many plant species with extrafloral nectaries, and is found nesting on both *H. laurifolia* in Sri Lanka and *H. vahliana* Wight in south India. Two undescribed species of ants were collected.

The following species were nesting within hollow internodes of *Humboldtia brunonis*. The species are arranged according to Bolton (1995).

Dolichoderinae

Tapinoma indicum Forel
Technomyrmex albipes (F. Smith)
Technomyrmex, prob. n. sp.

Formicinae

Camponotus compressus F.
Polyrhachis illaudata Walker
Polyrhachis, n. sp.

Myrmicinae

Crematogaster nilgirica Emery
Crematogaster prob. *travancorensis* Forel
Crematogaster sp. 1
Monomorium sp.
Tetramorium pacificum Mayr

Anthophoridae

Braunsapis sp., an undescribed bee, was found in 13 internodes out of 100 collected by KVK, and was also common in the KG collections. Both sexes and all stages of development were present. Given that the species has not been collected before, despite being a common associate of the tree, we assume that it does not venture far from the internodes. There was no evidence of the food used by this bee. This species is the same associated with *H. laurifolia* in Sri Lanka, however in Sri Lanka, *Braunsapis* sp. was only found in four internodes out of over 1300 sampled, while two wasps, *Krombeinictus nordenae* Leclercq, and *Crossocerus mukalanae* Leclercq were common. Neither of the wasps however, was collected from *H. brunonis* in India.

Bethylidae

A solitary, small, pale, bethylid pupa was removed from a single internode. These small wasps are parasitoids of Coleoptera and Lepidoptera, but no other organisms were noted in the internode.

Coleoptera

Cleridae

Numerous clerid larvae were collected from three internodes. In one, a single late stage predaceous larva was in an internode containing *Braunsapis* eggs. Another larva of the same species was found in an internode with a queen and worker *Crematogaster*, and approximately 30, 2mm long

clerid larvae were taken from another internode which lacked other organisms. Clerid larvae were also found associated with *Braunsapis* brood internodes in *H. laurifolia* from Sri Lanka.

Staphylinidae

A staphylinid larva was found in an internode without any evidence of prey, while a second larva was present in an internode which had a silken cap across the cavity above the larva.

Diptera

A dipterous pupa, with black legs, was attached to, and below, a flat closure disk. The cavity had mucus coating the walls. Three puparia of another diptera were removed from internodes, and 20 immature diptera were collected from a KG bulk sample.

Collembola

Collembola were found just within the entrance hole of three unoccupied internodes.

Orthoptera

Two Blattodae oothecae were found within an internode, and several adult cockroaches were found on trees.

Oligochaeta

Megascolecidae

Pale, approximately 2.5 cm long annelids were found in a number of internodes. These appeared similar to the undescribed, immature *Perionyx* sp. collected from *H. laurifolia,* but were adults in *H. brunonis.* This arboreal annelid is interesting in that the posterior one-third is curved and acts as a suction devise while the organism moves leech-like over a surface (Krombein et al. 1999).

Araneida

Salticidae

A small salticid, with some webbing, was removed from one internode.

Chilopoda

A single, small centipede was collected within an otherwise empty internode.

Discussion

The geologic history of India and Sri Lanka is one of intermittent connection by a land bridge, corresponding with various ice age fluctuations in ocean level. Fossil evidence indicates that numerous plant and animal species traveled this bridge, while others did not seem to make the transition, leading to a mixture of species common to both countries and also numerous endemics on the two land masses. The center of origin for *Humboldtia,* based on current species distribution, is within the Indian Western Ghats, but there is no fossil record indicating how far east, towards Sri Lanka, the genus might have once extended. Based on the fossil record, the southern portion of India was more highly forested in the past, and either *H. brunonis,* or some extinct ancestor, could have existed in close proximity to the Indian-Sri Lankan land bridge, making seed dispersal between the two land masses more probable than today. We do not envision a previous presence of other *Humboldtia* spp. on Sri Lanka with an extinction of all taxa except *H. laurifolia.* Likewise, given the wide range of environments presently occupied by *H. laurifolia,* and its current success within those environments, we do not think that this species once existed in India and then become extinct.

Morphologically, and within a taxonomic framework, *H. brunonis* and *H. laurifolia* are the most closely related species within the genus. Additionally, *H. brunonis* is the sole Indian *Humboldtia* species existing on the eastern side of the Western Ghats. Given the previous forestation of southern India, we hypothesize a seed dispersal event from the Indian subcontinent giving rise to what today is *H. laurifolia.*

If *H. brunonis* and *H. laurifolia* originated by speciation after a seed dispersal

event, rather than a vicariant separation of existing species, then the present associated invertebrates evolved their association with these *Humboldtia* spp. *in situ*, and the occupants found in the two tree species represent independent adaptations to a very similar domatium morphology and nectary location, on two separate land masses. Today, the two species exist in similar environments of wet evergreen to wet deciduous forests. These similar habitats probably forecast a somewhat similar invertebrate fauna from which the currently associated organisms evolved their relationship.

Of eleven inhabiting ant taxa, there is no dominant species on *H. brunonis*. *Technomyrmex albipes* is strongly dominant on *H. laurifolia* across habitats, but this ant is only an occasional occupant of *H. brunonis*. Conversely, *Crematogaster* was found nesting on almost every *H. brunonis* tree, comprising approximately 50% of the domatia inhabitants, but this genus was collected from only 13 internodes out of over 1,300 domatia sampled from *H. laurifolia* in Sri Lanka. *Tapinoma indicum* is a minute ant that was found nesting in two internodes on *H. brunonis*, and four domatia of *H. laurifolia*. All colonies were very strong with hundreds of workers and all stages of larvae and pupa in each internode. *Tapinoma indicum* occurs as a minor occupant in several ant plants in the old world and seems to be able to co-exist with a number of ant species, even in an adjacent domatium on *Humboldtia*, but never dominates all the domatia on even a small branch. The two species of *Polyrhachis* which nest within *H. brunonis* internodes contrast with *P. bugnioni*, associated with *H. laurifolia* in Sri Lanka, which binds leaves together to build its nests and so nests externally to the hollow internodes. *Polyrhachis gracilior* Forel binds leaves of *Humboldtia decurrens* in southern India, just as *P. bugnioni* does in Sri Lanka. Thus, *H. brunonis* is distinct from the other two myrmecophytic *Humboldtia* species in having its associated *Polyrhachis* species nesting within the hollow internodes rather than in leaf nests.

The other four ant species collected from *H. brunonis* domatia, *Camponotus compressus* F., *Monomorium* sp., *Tetramorium pacificum* Mayr, and *Technomyrmex* prob. n. sp., were collected from only one to several internodes each. Of these ant species, *T. pacificum* and the *Monomorium* species are identical to those from *H. laurifolia*, whereas *C. compressus* and *Technomyrmex* prob. n. sp. are unique to *H. brunonis* in our collections.

A major difference between *H. brunonis* and *H. laurifolia* is the lack of a wasp species being associated with *H. brunonis*, and a bee, *Braunsapis* sp. taking that place. A survey of the area to determine the availability of small wasps capable of using the internodes for nest sites would be very interesting, and surveys of other populations of *H. brunonis* in different environments may yet find wasps using the internodes. The bee *Braunsapis* sp. which is a minor inhabitant of *H. laurifolia* becomes a major inhabitant of *H. brunonis* possibly because of the absence of a competing wasp species. It is interesting that a bee or wasp is associated in a major way with both myrmecophytic *Humboldtia* species; this is not just an ant-associated tree.

Representatives of the beetle family Cleridae are associated with *Braunsapis*, probably as a brood predator, on both *H. brunonis* and *H. laurifolia*. However, the pseudoscorpion *Haplochernes warburgi*, a common ant predator on *H. laurifolia*, was absent from *H. brunonis*. Another common aggressive predator associated with numerous *H. laurifolia* internodes, a new Diptera (Keroplatidae) species, *Platyceridion edax* Chandler and Matile, is absent from our *H. brunonis* population. In fact, there seems to be no strong predator present in the Makut population despite a diverse ant fauna as potential prey. Instead, we find the few Cleridae, Staphylinidae, and diptera larvae to be the only possible predators.

Annelids, perhaps the same undescribed,

arboreal *Perionyx* species, are found in both *Humboldtia* species. In both cases the worms are nocturnal or exit the internodes during light daytime rains. We never found the worms associated with another invertebrate within an internode, but see no obvious method used to exclude competitors from the internodal space.

The population of *H. brunonis* sampled for this study did not possess either the species diversity or density of domatia occupancy found in *H. laurifolia*. Of the 100 *H. brunonis* internodes opened by KVK, 62% were occupied. Domatia occupancy on *H. laurifolia* was always above 90% for a given tree, and reached 100% on several bulk samples collected from single trees. We have reported on a single branch diversity of *H. laurifolia*, with 10 adjacent domatia possessing two ant species, *Technomyrmex albipes* and *Cataulacus taprobanae* F. Smith, the dipteran *Platyceridion edax*, and the wasp *Krombeinictus nordenae* Leclercq (Krombein et al. 1999). We did not find such single-branch diversity on *H. brunonis*, rather *Crematogaster* would usually dominate domatia along a branch, with one of the other invertebrates in single, random internodes.

A very preliminary examination of two small populations of *H. decurrens*, the third myrmecophytic species, in the southern Western Ghats of India, revealed three species of ants, *Polyrhachis gracilior* Forel, *Technomyrmex* prob. *brunneus* Forel, and an undescribed species of *Cataulacus*. This very southern species of *Humboldtia*, which prefers wetter habitats than *H. brunonis*, deserves a complete survey of its associated organisms.

It must be pointed out that of the eleven ant taxa collected from *H. brunonis*, a very common and easily accessible plant, two are new species. These discoveries, along with another undescribed ant collected from a small sample of hollow internodes of *H. decurrens* from southern India, and up to 11 new insect species across three Orders from *H. laurifolia* in Sri Lanka, point out how these cryptic plant cavities have never been collected, and how they harbor a unique fauna along with such common species as the ant *Technomyrmex albipes*.

Finally, it must be mentioned how important the genus *Humboldtia* is in harboring a unique series of organisms relating to local biodiversity. *Humboldtia laurifolia* from evergreen wet locations harbors at least 11 undescribed organisms, while a single population of *H. brunonis*, in a dryer environment, produced four undescribed species, and *H. decurrens*, one new species after vary limited collection. The role of *Humboldtia* and its hollow, self-opening internodes in harboring cryptic, rare species may be unparalleled among invertebrate-associated plants in south Asia.

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